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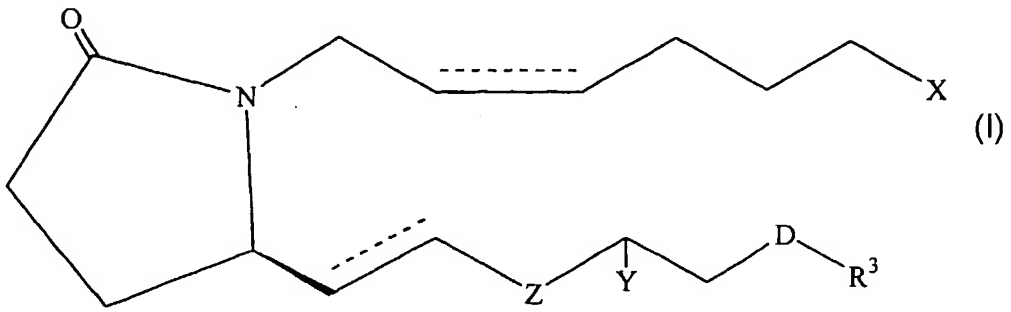
(54) Title: 8-AZAPROSTAGLANDIN ANALOGS AS AGENTS FOR LOWERING INTRAOCULAR PRESSURE



(I)

(57) Abstract: The present invention provides a method of treating ocular hypertension or glaucoma which comprises administering to an animal having ocular hypertension or glaucoma therapeutically effective amount of a compound represented by the general formula I; wherein X, Y, Z, D and R^3 are as defined in the specification.

WO 03/097596 A1

8-AZAPROSTAGLANDIN ANALOGS AS AGENTS FOR LOWERING INTRAOCULAR PRESSURE

Field of the Invention

The present invention relates 8-Azaprostaglandin analogues as potent ocular hypotensives that are particularly suited for the management of glaucoma.

Background of the Invention

Description of Related Art

Ocular hypotensive agents are useful in the treatment of a number of various ocular hypertensive conditions, such as post-surgical and post-laser trabeculectomy ocular hypertensive episodes, glaucoma, and as presurgical adjuncts.

Glaucoma is a disease of the eye characterized by increased intraocular pressure. On the basis of its etiology, glaucoma has been classified as primary or secondary. For example, primary glaucoma in adults (congenital glaucoma) may be either open-angle or acute or chronic angle-closure. Secondary glaucoma results from pre-existing ocular diseases such as uveitis, intraocular tumor or an enlarged cataract.

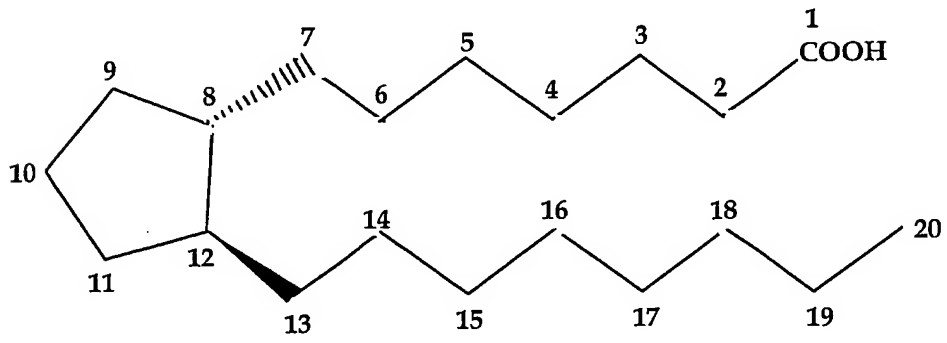
The underlying causes of primary glaucoma are not yet known. The increased intraocular tension is due to the obstruction of aqueous humor outflow. In chronic open-angle glaucoma, the anterior chamber and its anatomic structures appear normal, but drainage of the aqueous humor is impeded. In acute or chronic angle-closure glaucoma, the anterior chamber is shallow, the filtration angle is narrowed, and the iris may obstruct the trabecular meshwork at the entrance of the canal of Schlemm. Dilation of the pupil may push the root of the iris forward against the angle, and may produce pupilary block and thus precipitate an acute attack. Eyes

with narrow anterior chamber angles are predisposed to acute angle-closure glaucoma attacks of various degrees of severity.

Secondary glaucoma is caused by any interference with the flow of aqueous humor from the posterior chamber into the anterior chamber and subsequently, into the canal of Schlemm. Inflammatory disease of the anterior segment may prevent aqueous escape by causing complete posterior synechia in iris bombe, and may plug the drainage channel with exudates. Other common causes are intraocular tumors, enlarged cataracts, central retinal vein occlusion, trauma to the eye, operative procedures and intraocular hemorrhage.

Considering all types together, glaucoma occurs in about 2% of all persons over the age of 40 and may be asymptotic for years before progressing to rapid loss of vision. In cases where surgery is not indicated, topical b-adrenoreceptor antagonists have traditionally been the drugs of choice for treating glaucoma.

Certain eicosanoids and their derivatives have been reported to possess ocular hypotensive activity, and have been recommended for use in glaucoma management. Eicosanoids and derivatives include numerous biologically important compounds such as prostaglandins and their derivatives. Prostaglandins can be described as derivatives of prostanoic acid which have the following structural formula:



Various types of prostaglandins are known, depending on the structure and substituents carried on the alicyclic ring of the prostanoic acid skeleton. Further classification is based on the number of unsaturated bonds in the side chain indicated

by numerical subscripts after the generic type of prostaglandin [e.g. prostaglandin E_1 (PGE_1), prostaglandin E_2 (PGE_2)], and on the configuration of the substituents on the alicyclic ring indicated by α or β [e.g. prostaglandin $F_{2\alpha}$ ($PGF_{2\beta}$)].

Prostaglandins were earlier regarded as potent ocular hypertensives, however, evidence accumulated in the last decade shows that some prostaglandins are highly effective ocular hypotensive agents, and are ideally suited for the long-term medical management of glaucoma (see, for example, Bito, L.Z. Biological Protection with Prostaglandins, Cohen, M.M., ed., Boca Raton, Fla, CRC Press Inc., 1985, pp. 231-252; and Bito, L.Z., Applied Pharmacology in the Medical Treatment of Glaucomas Drance, S.M. and Neufeld, A.H. eds., New York, Grune & Stratton, 1984, pp. 477-505. Such prostaglandins include $PGF_{2\alpha}$, $PGF_{1\alpha}$, PGE_2, and certain lipid-soluble esters, such as C_1 to C_2 alkyl esters, e.g. 1-isopropyl ester, of such compounds.

Although the precise mechanism is not yet known experimental results indicate that the prostaglandin-induced reduction in intraocular pressure results from increased uveoscleral outflow [Nilsson et.al., Invest. Ophthalmol. Vis. Sci. (suppl), 284 (1987)].

The isopropyl ester of $PGF_{2\alpha}$ has been shown to have significantly greater hypotensive potency than the parent compound, presumably as a result of its more effective penetration through the cornea. In 1987, this compound was described as "the most potent ocular hypotensive agent ever reported" [see, for example, Bito, L.Z., Arch. Ophthalmol. 105, 1036 (1987), and Siebold et.al., Prodrug 5 3 (1989)].

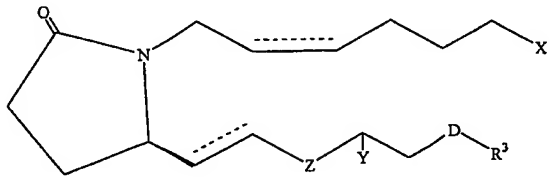
Whereas prostaglandins appear to be devoid of significant intraocular side effects, ocular surface (conjunctival) hyperemia and foreign-body sensation have been consistently associated with the topical ocular use of such compounds, in particular $PGF_{2\alpha}$ and its prodrugs, e.g., its 1-isopropyl ester, in humans. The clinical potentials of prostaglandins in the management of conditions associated with increased ocular pressure, e.g. glaucoma are greatly limited by these side effects.

In a series of co-pending United States patent applications assigned to Allergan, Inc. prostaglandin esters with increased ocular hypotensive activity accompanied with no or substantially reduced side-effects are disclosed. The co-pending USSN 596,430 (filed 10 October 1990, now U.S. Patent 5,446,041), relates to certain 11-acyl-prostaglandins, such as 11-pivaloyl, 11-acetyl, 11-isobutyryl, 11-valeryl, and 11-isovaleryl $PGF_{2\alpha}$. Intraocular pressure reducing 15-acyl prostaglandins are disclosed in the co-pending application USSN 175,476 (filed 29 December 1993). Similarly, 11,15- 9,15 and 9,11-diesters of prostaglandins, for example 11,15-dipivaloyl $PGF_{2\alpha}$ are known to have ocular hypotensive activity. See the co-pending patent applications USSN Nos. 385,645 (filed 07 July 1989, now U.S. Patent 4,994,274), 584,370 (filed 18 September 1990, now U.S. Patent 5,028,624) and 585,284 (filed 18 September 1990, now U.S. Patent 5,034,413). The disclosures of all of these patent applications are hereby expressly incorporated by reference.

8-Azaprostaglandin analogs are disclosed in PCT Patent Application WO 01/46140 A1.

Summary of the Invention

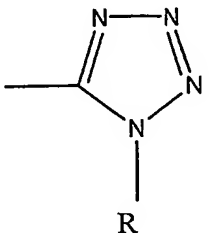
The present invention concerns a method of treating ocular hypertension which comprises administering to a mammal having ocular hypertension a therapeutically effective amount of a compound of formula I

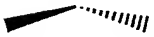


wherein hatched lines represent the α configuration, a triangle represents the β configuration, a wavy line represents either the α configuration or the β configuration and a dotted line represents the presence or absence of a double bond;

D represents a covalent bond or CH_2 , O, S or NH;

X is CO_2R, $CONR_2$, CH_2OR, $P(O)(OR)_2$, $CONRSO_2R$, $SONR_2$ or



Y is H  OR^1, R^1O  H, H   OR^1 or O;

Z is CH_2 or a covalent bond;

R is H or R^2;

R^1 is H, R^2 , phenyl, or COR^2;

R^2 is C_1-C_5 lower alkyl or alkenyl and R^3 is selected from the group consisting of R^2, phenyl, thienyl, furanyl, pyridyl, benzothienyl, benzofuranyl, naphthyl, or substituted derivatives thereof, wherein the substituents maybe selected from the group consisting of C_1-C_5 alkyl, halogen, CF_3, CN, NO_2, NR_2, CO_2R and OR .

In a still further aspect, the present invention relates to a pharmaceutical product, comprising

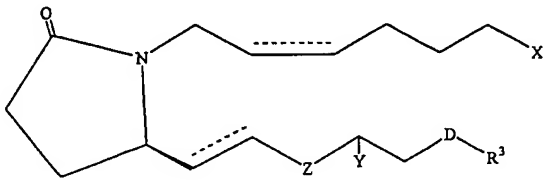
a container adapted to dispense its contents in a metered form; and

an ophthalmic solution therein, as hereinabove defined.

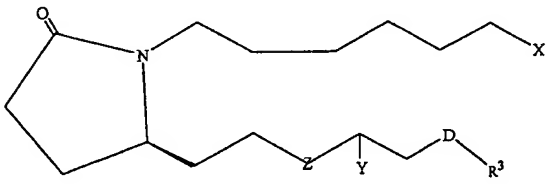
Finally, certain of the compounds represented by the above formula, disclosed below and utilized in the method of the present invention are novel and unobvious.

Detailed Description of the Invention

The present invention relates to the use of 8-Azaprostaglandin analogs as ocular hypotensives. The compounds used in accordance with the present invention are encompassed by the following structural formula I:



The preferred group of the compounds of the present invention includes compounds that have the following structural formula II.



In the above formulae, the substituents and symbols are as hereinabove defined.

In the above formulae:

Preferably D represents a covalent bond or is CH_2; more preferably D is CH_2.

Preferably Z represents a covalent bond.

Preferably R is H or C_1-C_5 lower alkyl.

Preferably R^1 is H.

Preferably R^3 is selected from the group consisting of phenyl and monosubstituted derivatives thereof, e.g. chloro and trifluoromethyl phenyl.

Preferably X is CO_2R and more preferably R is selected from the group consisting of H and ethyl.

The above compounds of the present invention may be prepared by methods that are known in the art or according to the working examples below. The compounds, below, are especially preferred representative, of the compounds of the present invention.

7-[2S-[3R-Hydroxy-4-(phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[3R-Hydroxy-4-(phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester;

7-[2S-[4-(3-Chloro-phenyl)-3R-hydroxy-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[4-(3-Chloro-phenyl)-3R-hydroxy-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester;

7-[2S-[3R-Hydroxy-4-(3-trifluoromethyl-phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[3R-Hydroxy-4-(3-trifluoromethyl-phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester;

7-[2S-[3-Oxo-4-(phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[3-Oxo-4-(phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester;

7-[2S-[4-(3-Chloro-phenyl)-3-oxo-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[4-(3R-Chloro-phenyl)-3-oxo-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester;

7-[2S-[3-Oxo-4-(3-trifluoromethyl-phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[3-Oxo -4-(3-trifluoromethyl-phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester;

7-[2S-[3R-Hydroxy-4-(chloro-phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[3R-Hydroxy-4-(chloro-phenyl)-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[3R-Hydroxy-4-(chlorophenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester;

7-[2S-[3R-Hydroxy-4-(chlorophenyl)-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester;

7-[2S-[4-(3-Chloro-phenyl)-3-oxo-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[4-(3-Chloro-phenyl)-3-oxo-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[4-(3-Chloro-phenyl)-3-oxo-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester;

7-[2S-[4-(3-Chloro-phenyl)-3-oxo-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester;

7-[2S-[3R-Hydroxy-4-(3-trifluoromethyl-phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[3R-Hydroxy-4-(3-trifluoromethyl-phenyl)-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[3R-Hydroxy-4-(3-trifluoromethyl-phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester;

7-[2S-[3R-Hydroxy-4-(3-trifluoromethyl-phenyl)-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester;

7-[2S-[3-Oxo-4-(trifluormethylphenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[3-Oxo-4-(trifluormethylphenyl)-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[3-Oxo-4-(trifluoromethylphenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester;

7-[2S-[3-Oxo-4-(trifluoromethylphenyl)-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester;

7-[2S-[3R-Hydroxy-(4-phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[3R-Hydroxy-(4-phenyl)-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[3R-Hydroxy-(4-phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester;

7-[2S-[3R-Hydroxy-(4-phenyl)-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester;

7-[2S-[3-Oxo-4-(phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[3-Oxo-4-(phenyl)-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[3-Oxo-4-(phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester and

7-[2S-[3-Oxo-4-(phenyl)-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester.

Pharmaceutical compositions may be prepared by combining a therapeutically effective amount of at least one compound according to the present invention, or a pharmaceutically acceptable acid addition salt thereof, as an active ingredient, with conventional ophthalmically acceptable pharmaceutical excipients, and by preparation of unit dosage forms suitable for topical ocular use. The therapeutically efficient amount typically is between about 0.0001 and about 5% (w/v), preferably about 0.001 to about 1.0% (w/v) in liquid formulations.

For ophthalmic application, preferably solutions are prepared using a physiological saline solution as a major vehicle. The pH of such ophthalmic solutions should preferably be maintained between 6.5 and 7.2 with an appropriate buffer system. The formulations may also contain conventional, pharmaceutically acceptable preservatives, stabilizers and surfactants.

Preferred preservatives that may be used in the pharmaceutical compositions of the present invention include, but are not limited to, benzalkonium chloride, chlorobutanol, thimerosal, phenylmercuric acetate and phenylmercuric nitrate. A

preferred surfactant is, for example, Tween 80. Likewise, various preferred vehicles may be used in the ophthalmic preparations of the present invention. These vehicles include, but are not limited to, polyvinyl alcohol, povidone, hydroxypropyl methyl cellulose, poloxamers, carboxymethyl cellulose, hydroxyethyl cellulose and purified water.

Tonicity adjustors may be added as needed or convenient. They include, but are not limited to, salts, particularly sodium chloride, potassium chloride, mannitol and glycerin, or any other suitable ophthalmically acceptable tonicity adjustor.

Various buffers and means for adjusting pH may be used so long as the resulting preparation is ophthalmically acceptable. Accordingly, buffers include acetate buffers, citrate buffers, phosphate buffers and borate buffers. Acids or bases may be used to adjust the pH of these formulations as needed.

In a similar vein, an ophthalmically acceptable antioxidant for use in the present invention includes, but is not limited to, sodium metabisulfite, sodium thiosulfate, acetylcysteine, butylated hydroxyanisole and butylated hydroxytoluene.

Other excipient components which may be included in the ophthalmic preparations are chelating agents. The preferred chelating agent is edentate disodium, although other chelating agents may also be used in place or in conjunction with it.

The ingredients are usually used in the following amounts:

Ingredient	Amount (% w/v)
active ingredient	about 0.001-5
preservative	0-0.10
vehicle	0-40
tonicity adjustor	1-10
buffer	0.01-10
pH adjustor	q.s. pH 4.5-7.5
antioxidant	as needed
surfactant	as needed

purified water	as needed to make 100%

The actual dose of the active compounds of the present invention depends on the specific compound, and on the condition to be treated; the selection of the appropriate dose is well within the knowledge of the skilled artisan.

The ophthalmic formulations of the present invention are conveniently packaged in forms suitable for metered application, such as in containers equipped with a dropper, to facilitate the application to the eye. Containers suitable for dropwise application are usually made of suitable inert, non-toxic plastic material, and generally contain between about 0.5 and about 15 ml solution.

This invention is further illustrated by the following non-limiting Examples.

Example 1

7-[2S-[3R-Hydroxy-4-(chloro-phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid

Example 1a

7-[2S-[3R-Hydroxy-4-(chloro-phenyl)-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid

Example 2

7-[2S-[3R-Hydroxy-4-(chlorophenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester

Example 2a

7-[2S-[3R-Hydroxy-4-(chlorophenyl)-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester

Example 3

7-[2S-[4-(3-Chloro-phenyl)-3-oxo-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid

Example 3a

7-[2S-[4-(3-Chloro-phenyl)-3-oxo-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid

Example 4

7-[2S-[4-(3-Chloro-phenyl)-3-oxo-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester

Example 4a

7-[2S-[4-(3-Chloro-phenyl)-3-oxo-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester

Example 5

7-[2S-[3R-Hydroxy-4-(3-trifluoromethyl-phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid

Example 5a

7-[2S-[3R-Hydroxy-4-(3-trifluoromethyl-phenyl)-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid

Example 6

7-[2S-[3R-Hydroxy-4-(3-trifluoromethyl-phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester

Example 6a

7-[2S-[3R-Hydroxy-4-(3-trifluoromethyl-phenyl)-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester

Example 7

7-[2S-[3-Oxo-4-(trifluormethylphenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid

Example 7a

7-[2S-[3-Oxo-4-(trifluormethylphenyl)-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid

Example 8

7-[2S-[3-Oxo-4-(trifluoromethylphenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester

Example 8a

7-[2S-[3-Oxo-4-(trifluoromethylphenyl)-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester

The compounds of Examples 1 through 8a are made according to the methods disclosed in Examples 1 and 2 of published PCT Patent Application WO 01/46140, which is hereby incorporated by reference herein.

Example 9

7-[2S-[3R-Hydroxy-(4-phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid

Example 9a

7-[2S-[3R-Hydroxy-(4-phenyl)-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid

Example 10

7-[2S-[3R-Hydroxy-(4-phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester

Example 10a

7-[2S-[3R-Hydroxy-(4-phenyl)-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester

Example 11

7-[2S-[3-Oxo-4-(phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid

Example 11a

7-[2S-[3-Oxo-4-(phenyl)-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid

Example 12

7-[2S-[3-Oxo-4-(phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester

Example 12a

7-[2S-[3-Oxo-4-(phenyl)-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester

The compounds of Examples 9 through 12a are made by methods analogous to the methods used to make the compounds of Examples 1 through 8, with [3-(phenyl)-2-oxo-propyl]-phosphonic acid dimethyl ester replacing [3-(3-chloro-phenyl)-2-oxo-propyl]-phosphonic acid dimethyl ester.

These compounds are tested for in vitro activity as described below and the results given in the Tables.

TABLE 1
8-Azaprostaglandin Analogs - Functional Data

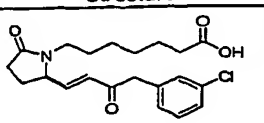
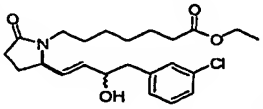
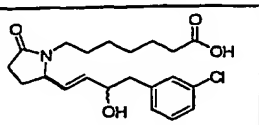
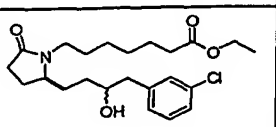
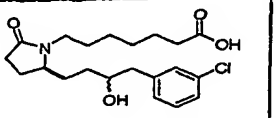
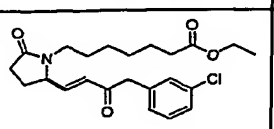
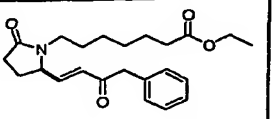
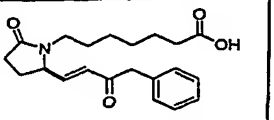
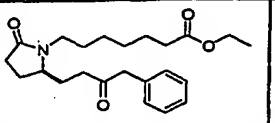
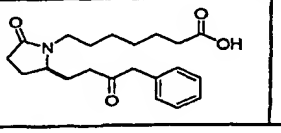
Example#	Structure	hFP	hEP_1	hEP_2	hEP_{3A}	hEP_4	hTP	hIP	hDP
3a		NA	hit	NA	324	54	$>10^4$	NA	NA
2a		NA	NA	NA	NA	21	NA	NA	NA
1a		NA	hit	NA	324	0.02	$>10^4$	NA	NA
2		NA	NA	NA	NA	65	NA	NA	NA
1		NA	$>10^4$	NA	608	0.7	$>10^4$	NA	NA
4a		NA	NA	NA	NA	$>10^4$	NA	NA	NA
12a		NA	NA	NA	NA	$>10^4$	NA	NA	NA
11a		NA	NA	NA	hit	29	$>10^4$	NA	NA
12		NA	NA	NA	NA	$>10^4$	NA	NA	NA
11		NA	NA	NA	NA	193	NA	NA	NA

TABLE 1
8-Azaprostaglandin Analogs - Functional Data

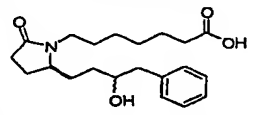
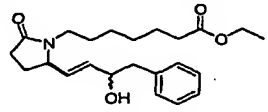
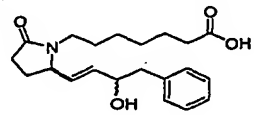
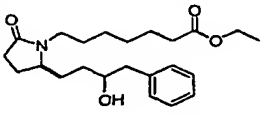
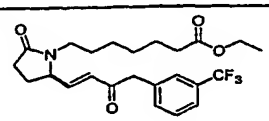
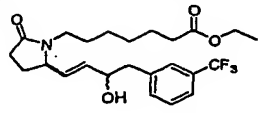
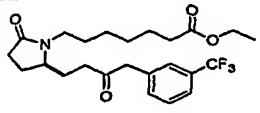
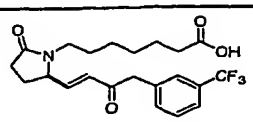
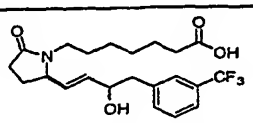
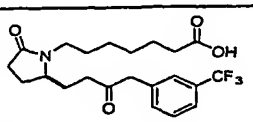
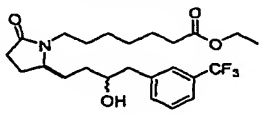
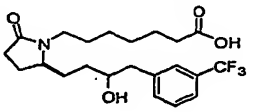
Example#	Structure	hFP	hEP_1	hEP_2	hEP_{3A}	hEP_4	hTP	hIP	hDP
9		NA	NA	NA	$>10^4$	2.4	NA	NA	NA
10a		NA	NA	NA	NA	368	NA	NA	NA
9a		NA	NA	NA	NA	0.9	NA	NA	NA
10		NA	NA	NA	NA	1023	NA	NA	NA
8a		NA	NA	NA	$>10^4$	$>10^4$	NA	NA	NA
6a		NA	NA	NA	$>10^4$	26	NA	$>10^4$	NA
8		NA	NA	NA	NA	7161	NA	NA	NA
7a		NA	$>10^4$	NA	hit	86	NA	NA	NA
5a		NA	NA	NA	hit	0.4	NA	NA	NA
7		NA	$>10^4$	NA	hit	551	$>10^4$	NA	NA

TABLE 1
8-Azaprostaglandin Analogs - Functional Data

Example#	Structure	hFP	hEP_1	hEP_2	hEP_{3A}	hEP_4	hTP	hIP	hDP
6		NA	NA	NA	NA	111	NA	NA	NA
5		NA	NA	NA	hit	0.4	NA	NA	NA

All data are EC_{50} in nM

Table 2
8-Azaprostaglandin Analogs - Radioligand Binding Data

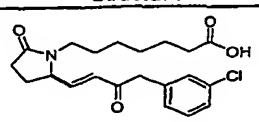
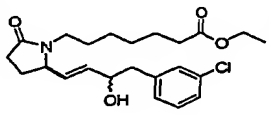
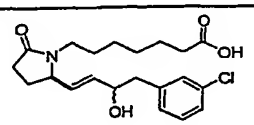
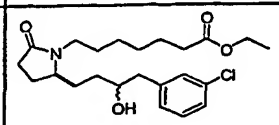
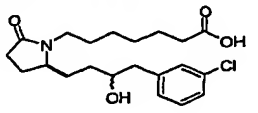
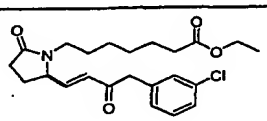
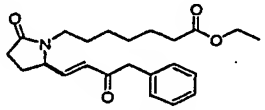
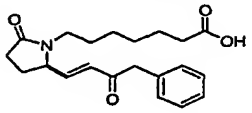
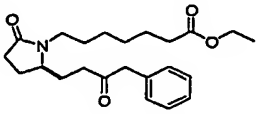
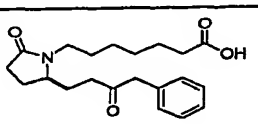
Example#	Structure	hFP	hEP_1	hEP_2	hEP_{3D}	hEP_4	hTP	hIP
3a				NA		300		
2a				NA		300		
1a				$>10^4$		0.4		
2				NA		1000		
1				5800		12		
4a				NA		$>10^4$		
12a				NA		$>10^4$		
11a				NA		300		
12				NA		8900		
11				NA		1500		

Table 2
8-Azaprostaglandin Analogs - Radioligand Binding Data

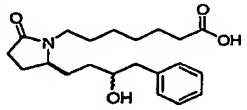
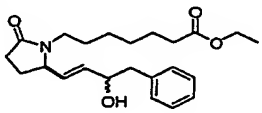
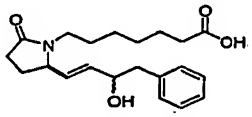
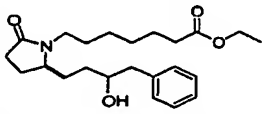
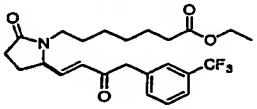
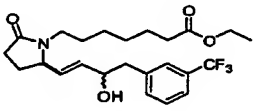
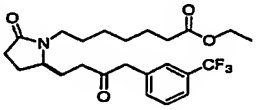
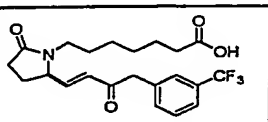
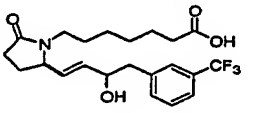
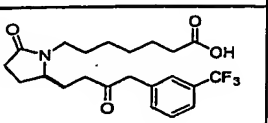
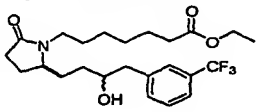
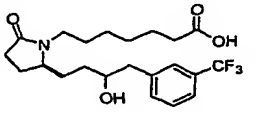
Example#	Structure	hFP	hEP_1	hEP_2	hEP_{3D}	hEP_4	hTP	hIP
9				NA		18		
10a				NA		600		
9a				NA		9		
10								
8a				NA		$>10^4$		
6a				NA		200		
8				NA		$>10^4$		
7a				$>10^4$		500		
5a				NA		5		
7				NA		2200		

Table 2
8-Azaprostaglandin Analogs - Radioligand Binding Data

Example#	Structure	hFP	hEP_1	hEP_2	hEP_{3D}	hEP_4	hTP	hIP
6				NA		1200		
5				NA		17		

values are IC_{50} in nM

HUMAN RECOMBINANT EP_1, EP_2, EP_3, EP_4, FP, TP, IP and DP RECEPTORS: STABLE TRANSFECTANTS.

Plasmids encoding the human EP_1, EP_2, EP_3, EP_4, FP, TP, IP and DP receptors were prepared by cloning the respective coding sequences into the eukaryotic expression vector pCEP4 (Invitrogen). The pCEP4 vector contains an Epstein Barr virus (EBV) origin of replication, which permits episomal replication in primate cell lines expressing EBV nuclear antigen (EBNA-1). It also contains a hygromycin resistance gene that is used for eukaryotic selection. The cells employed for stable transfection were human embryonic kidney cells (HEK-293) that were transfected with and express the EBNA-1 protein. These HEK-293-EBNA cells (Invitrogen) were grown in medium containing Geneticin (G418) to maintain expression of the EBNA-1 protein. HEK-293 cells were grown in DMEM with 10% fetal bovine serum (FBS), 250 $\mu g\ ml^{-1}$ G418 (Life Technologies) and 200 $\mu g\ ml^{-1}$ gentamicin or penicillin/streptomycin. Selection of stable transfectants was achieved with 200$\mu g\ ml^{-1}$ hygromycin, the optimal concentration being determined by previous hygromycin kill curve studies.

For transfection, the cells were grown to 50-60% confluency on 10 cm plates. The plasmid pCEP4 incorporating cDNA inserts for the respective human prostanoid receptor (20 μg) was added to 500 μl of 250 mM $CaCl_2$. HEPES buffered saline x 2 (2 x HBS, 280 mM NaCl, 20 mM HEPES acid, 1.5 mM Na_2HPO_4, pH 7.05 – 7.12) was then added dropwise to a total of 500 μl, with continuous vortexing at room temperature. After 30 min, 9 ml DMEM were added to the mixture. The DNA/DMEM/calcium phosphate mixture was then added to the cells, which had been previously rinsed with 10 ml PBS. The cells were then incubated for 5 hr at 37° C in humidified 95% air/5% CO_2. The calcium phosphate solution was then removed and the cells were treated with 10% glycerol in DMEM for 2 min. The glycerol solution was then replaced by DMEM with 10% FBS. The

cells were incubated overnight and the medium was replaced by DMEM/10% FBS containing 250 μg ml^{-1} G418 and penicillin/streptomycin. The following day hygromycin B was added to a final concentration of 200 μg ml^{-1}.

Ten days after transfection, hygromycin B resistant clones were individually selected and transferred to a separate well on a 24 well plate. At confluence each clone was transferred to one well of a 6 well plate, and then expanded in a 10 cm dish. Cells were maintained under continuous hygromycin selection until use.

RADIOLIGAND BINDING

Radioligand binding studies on plasma membrane fractions prepared for cells stably transfected with the cat or human receptor were performed as follows. Cells washed with TME buffer were scraped from the bottom of the flasks and homogenized for 30 sec using a Brinkman PT 10/35 polytron. TME buffer was added as necessary to achieve a 40 ml volume in the centrifuge tubes. TME is comprised of 50 mM TRIS base, 10 mM $MgCl_2$, 1mM EDTA; pH 7.4 is achieved by adding 1 N HCl. The cell homogenate was centrifuged at 19,000 rpm for 20-25 min at 4°C using a Beckman Ti-60 or Tι-70 rotor. The pellet was then resuspended in TME buffer to provide a final protein concentration of 1 mg/ml, as determined by Bio-Rad assay. Radioligand binding assays were performed in a 100 μl or 200 μl volume.

The binding of [^{3}H](N) PGE_2 (specific activity 165 Ci/mmol) was determined in duplicate and in at least 3 separate experiments. Incubations were for 60 min at 25° C and were terminated by the addition of 4 ml of ice-cold 50 mM TRIS-HC1 followed by rapid filtration through Whatman GF/B filters and three additional 4 ml washes in a cell harvester (Brandel). Competition studies were performed using a final concentration of 2.5 or 5 nM [^{3}H](N) PGE_2 and non-specific binding was determined with 10^{-5} M unlabelled PGE_2.

For radioligand binding on the transient transfectants, plasma membrane fraction preparation was as follows. COS-7 cells were washed with TME buffer,

scraped from the bottom of the flasks, and homogenized for 30 sec using a Brinkman PT 10/35 polytron. TME buffer was added to achieve a final 40 ml volume in the centrifuge tubes. The composition of TME is 100 mM TRIS base, 20 mM $MgCl_2$, 2M EDTA; 10N HCl is added to achieve a pH of 7.4.

The cell homogenate was centrifuged at 19000 rpm for 20 min at 4°C using a Beckman Ti-60 rotor. The resultant pellet was resuspended in TME buffer to give a final 1 mg/ml protein concentration, as determined by Biorad assay. Radioligand binding assays were performed in a 200 µl volume.

The binding of [^{3}H] PGE_2 (specific activity 165 Ci or mmol $^{-1}$) at EP_{3D}, receptors and [^{3}H]-SQ29548 (specific activity 41.5 Ci mmol^{-1}) at TP receptors were determined in duplicate in at least three separate experiments. Radiolabeled PGE_2 was purchased from Amersham, radiolabeled SQ29548 was purchased from New England Nuclear. Incubations were for 60 min at 25°C and were terminated by the addition of 4 ml of ice-cold 50 mM TRIS-HCl, followed by rapid filtration through Whatman GF/B filters and three additional 4 ml washes in a cell harvester (Brandel). Competition studies were performed using a final concentration of 2.5 or 5 nM [^{3}H]-PGE_2, or 10 nM [^{3}H]-SQ 29548 and non-specific binding determined with 10 µM of the respective unlabeled prostanoid. For all radioligand binding studies, the criteria for inclusion were >50% specific binding and between 500 and 1000 displaceable counts or better.

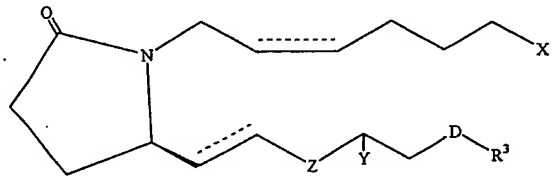
The effects of the compounds of this invention on intraocular pressure may be measured as follows. The compounds are prepared at the desired concentrations in a vehicle comprising 0.1% polysorbate 80 and 10 mM TRIS base. Dogs are treated by administering 25 µl to the ocular surface, the contralateral eye receives vehicle as a control. Intraocular pressure is measured by applanation pneumatonometry. Dog intraocular pressure is measured immediately before drug administration and at 6 hours thereafter.

The compounds of this invention are useful in lowering elevated intraocular pressure in mammals, e.g. humans.

The foregoing description details specific methods and compositions that can be employed to practice the present invention, and represents the best mode contemplated. However, it is apparent for one of ordinary skill in the art that further compounds with the desired pharmacological properties can be prepared in an analogous manner, and that the disclosed compounds can also be obtained from different starting compounds via different chemical reactions. Similarly, different pharmaceutical compositions may be prepared and used with substantially the same result. Thus, however detailed the foregoing may appear in text, it should not be construed as limiting the overall scope hereof; rather, the ambit of the present invention is to be governed only by the lawful construction of the appended claims.

CLAIMS

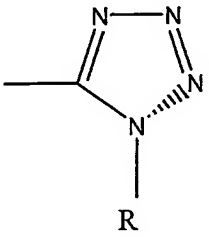
1. A method of treating ocular hypertension or glaucoma which comprises administering to an animal having ocular hypertension or glaucoma a therapeutically effective amount of a compound represented by the general formula I;



wherein hatched lines represent the α configuration, a triangle represents the β configuration, a wavy line represents either the α configuration or the β configuration and a dotted line represents the presence or absence of a double bond;

D represents a covalent bond or CH_2 , O, S or NH;

X is CO_2R, $CONR_2$, CH_2OR, $P(O)(OR)_2$, $CONRSO_2R$, $SONR_2$ or



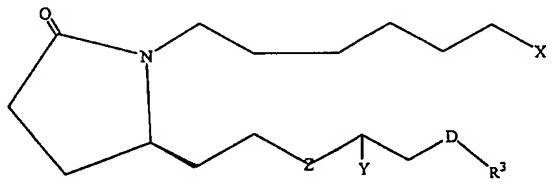
Z is CH_2 or a covalent bond;

R is H or R^2;

R^1 is H, R^2 , phenyl, or COR^2;

R^2 is C_1-C_5 lower alkyl or alkenyl and R_3 is selected from the group consisting of R^2, phenyl, thienyl, furanyl, pyridyl, benzothienyl, benzofuranyl, naphthyl, or substituted derivatives thereof, wherein the substituents maybe selected from the group consisting of C_1-C_5 alkyl, halogen, CF_3, CN, NO_2, NR_2, CO_2R and OR .

2. The method according to claim 1 wherein said compound is represented by the general formula II;



3. The method of claim 1 wherein Z represents a covalent bond.

4. The method of claim 1 wherein D is CH_2.

5. The method of claim 1 wherein X is CO_2 R.

6. The method of claim 5 wherein R is selected from the group consisting of H and ethyl.

7. The method of claim 5 wherein R is H, or C_1-C_5 alkyl.

8. The method of claim 1 wherein R_1 is H.

9. The method of claim 1 wherein R^3 is selected from the group consisting of phenyl, chlorophenyl and trifluoromethylphenyl.

10. The method of claim 1 wherein said compound is selected from the group consisting of

7-[2S-[3R-Hydroxy-4-(phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[3R-Hydroxy-4-(phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester;

7-[2S-[4-(3-Chloro-phenyl)-3R-hydroxy-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[4-(3-Chloro-phenyl)-3R-hydroxy-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester;

7-[2S-[3R-Hydroxy-4-(3-trifluoromethyl-phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[3R-Hydroxy-4-(3-trifluoromethyl-phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester;

7-[2S-[3-Oxo-4-(phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[3-Oxo-4-(phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester;

7-[2S-[4-(3-Chloro-phenyl)-3-oxo-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[4-(3R-Chloro-phenyl)-3-oxo-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester;

7-[2S-[3-Oxo-4-(3-trifluoromethyl-phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[3-Oxo -4-(3-trifluoromethyl-phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester;

7-[2S-[3R-Hydroxy-4-(chloro-phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[3R-Hydroxy-4-(chloro-phenyl)-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[3R-Hydroxy-4-(chlorophenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester;

7-[2S-[3R-Hydroxy-4-(chlorophenyl)-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester;

7-[2S-[4-(3-Chloro-phenyl)-3-oxo-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[4-(3-Chloro-phenyl)-3-oxo-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[4-(3-Chloro-phenyl)-3-oxo-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester;

7-[2S-[4-(3-Chloro-phenyl)-3-oxo-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester;

7-[2S-[3R-Hydroxy-4-(3-trifluoromethyl-phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[3R-Hydroxy-4-(3-trifluoromethyl-phenyl)-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[3R-Hydroxy-4-(3-trifluoromethyl-phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester;

7-[2S-[3R-Hydroxy-4-(3-trifluoromethyl-phenyl)-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester;

7-[2S-[3-Oxo-4-(trifluormethylphenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[3-Oxo-4-(trifluormethylphenyl)-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[3-Oxo-4-(trifluoromethylphenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester;

7-[2S-[3-Oxo-4-(trifluoromethylphenyl)-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester;

7-[2S-[3R-Hydroxy-(4-phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[3R-Hydroxy-(4-phenyl)-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[3R-Hydroxy-(4-phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester;

7-[2S-[3R-Hydroxy-(4-phenyl)-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester;

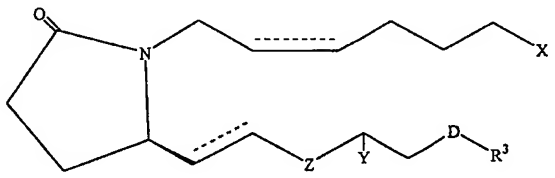
7-[2S-[3-Oxo-4-(phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[3-Oxo-4-(phenyl)-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[3-Oxo-4-(phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester and

7-[2S-[3-Oxo-4-(phenyl)-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester.

11. An ophthalmic solution comprising a therapeutically effective amount of a compound represented by the general Formula 1

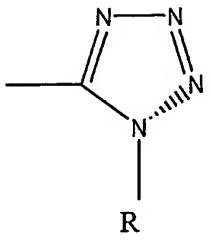


wherein hatched lines represent the α configuration, a triangle represents the β configuration, a wavy line represents the α configuration or the β configuration and a dotted line represents the presence or absence of a double bond;

D represents a covalent bond or CH_2 , O, S or NH;

X is C

O_2R, $CONR_2$, CH_2OR, $P(O)(OR)_2$, $CONRSO_2R$ $SONR_2$ or



Z is CH_2 or a covalent bond;

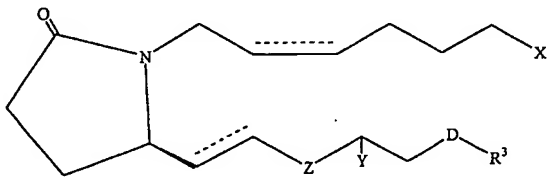
R is H or R^2;

R^1 is H, R^2 , phenyl, or COR^2;

R^2 is C_1-C_5 lower alkyl or alkenyl and R_3 is selected from the group consisting of R^2, phenyl, thienyl, furanyl, pyridyl, benzothienyl, benzofuranyl, naphthyl or substituted derivatives thereof, wherein the substituents maybe selected from the group consisting of C_1-C_5 alkyl, halogen, CF_3, CN, NO_2, NR_2, CO_2R and OR in admixture with a non-toxic, ophthalmically acceptable liquid vehicle, packaged in a container suitable for metered application.

12. A pharmaceutical product, comprising a container adapted to dispense the contents of said container in metered form; and an ophthalmic solution according to claim 11 in said container.

13. A compound useful for treating ocular hypertension or glaucoma which comprises a compound represented by the general formula I;



wherein hatched lines represent the α configuration, a triangle represents the β configuration, a wavy line represents either the α configuration or the β configuration and a dotted line represents the presence or absence of a double bond;

D represents a covalent bond or CH_2 , O, S or NH;

X is $CONR_2$, CH_2OR, $P(O)(OR)_2$, $CONRSO_2R$ or $SONR_2$;

Z is CH_2 or a covalent bond;

R is H or R^2;

R^1 is H, R^2 , phenyl, or COR^2;

R^2 is C_1-C_5 lower alkyl or alkenyl and R_3 is selected from the group consisting of R^2, phenyl, thienyl, furanyl, pyridyl, benzothienyl, benzofuranyl, naphthyl, or substituted derivatives thereof, wherein the substituents maybe selected from the group consisting of C_1-C_5 alkyl, halogen, CF_3, CN, NO_2, NR_2, CO_2R and OR .

14. A compound selected from the group consisting of

7-[2S-[3R-Hydroxy-4-(phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[3R-Hydroxy-4-(phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester;

7-[2S-[3-Oxo-4-(phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[3-Oxo-4-(phenyl)-butyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester;

7-[2S-[3R-Hydroxy-(4-phenyl)-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid;

7-[2S-[3R-Hydroxy-(4-phenyl)-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester;

7-[2S-[3-Oxo-4-(phenyl)-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid and

7-[2S-[3-Oxo-4-(phenyl)-but-1-enyl]-5-oxo-pyrrolidin-1-yl]-heptanoic acid, ethyl ester.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 03/13300

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D207/26 A61K31/4015 A61P27/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
CHEM ABS Data, EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE 27 35 904 A (PFIZER) 9 February 1978 (1978-02-09) examples 7,14	14
Y	page 51, line 1 -page 52, line 21; claims 1,13,31; examples 5-10	1-13
Y	EP 1 110 949 A (PFIZER PROD INC) 27 June 2001 (2001-06-27) cited in the application page 3, line 28 -page 4, line 1; claims 5-12; examples	1-14
Y	DE 25 28 664 A (HOECHST AG) 13 January 1977 (1977-01-13) page 37, line 1 - line 5; claims 1,27,28; examples 2CI1.,2CI4.,2CI5.,2BIII1.,2AV1.,6BI1.,6BI3.6BI7...	1-14

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 August 2003	27/08/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hanisch, I

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 03/13300

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 00 38667 A (ALCON LAB INC ;KLIMKO PETER G (US); SHARIF NAJAM A (US); GRIFFIN B) 6 July 2000 (2000-07-06) page 10, line 6 - line 10; claims 1,8; table 1	1-14
Y	EP 0 410 786 A (ALLERGAN INC) 30 January 1991 (1991-01-30) cited in the application page 3, line 6 - line 28; claims 1,6,10-12	1-14

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 1 and 13 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.1

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 03/13300

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 03/13300

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Internat Application No
PCT/US 03/13300

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